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COMMENTARY

Bloody *coli*: a Gene Cocktail in *Escherichia coli* O104:H4

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ABSTRACT A recent study published in *mBio* [Y. H. Grad et al., *mBio* 4(1):e00452-12, 2013] indicates that a rapid introgressive evolution has occurred in *Escherichia coli* O104:H4 by sequential acquisition of foreign genetic material involving pathogenicity traits. O104 genetic promiscuity cannot be readily explained by high population sizes. However, extensive interactions leading to cumulative assemblies of pathogenicity genes might be assured by small *K*-strategist populations exploiting particular intestinal niches. Next-generation sequencing technologies will be critical to detect particular "gene cocktails" as potentially pathogenic ensembles and to predict the risk of future outbreaks.

The cell is a unit of interaction. Bacterial cells from the species *Escherichia coli* specifically interact with the lower gastrointestinal tracts (including the microbiotas) of animals and humans, a relationship resulting from a long-term coevolutionary process that has shaped the well-defined *E. coli* core genome. We fully agree with the proposal that *E. coli* should be treated as a single microorganism, in spite of the fact that strains are often classified according to their intestinal pathotypes, including as enteropathogenic, enterotoxigenic, enterohemorrhagic, enteroaggregative, enteroinvasive, adherent-invasive (1), and, in the case of O104:H4, enteroaggregative hemorrhagic *E. coli* (EAHEC). All of these *E. coli* pathotypes have essentially the same core genome (comprising about 2,000 genes) maintained by vertical descent. During the course of *E. coli*'s evolution, a basic type of the *E. coli* genome seems to have been complemented by the acquisition by horizontal transfer of different adaptive (including "pathogenic") genes, genes that are propagated by introgressive descent (2). The outcome is the emergence of a variety of strains with different colonization or pathogenic abilities. From this perspective, it may be inappropriate to link the term "pathogenic" to particular serotypes or *E. coli* multilocus sequence types (MLSTs). Classic "pathogenic types" might correspond to those types where the acquisition of pathogenicity traits (PTs) has been documented with particular frequency. However, not all *E. coli* serotypes or sequence types (STs) are equally distributed in all habitats, indicating that a number of noncore genes have evolved to provide different degrees of ecological specificity, eventually leading to some kind of ecological barrier for intraspecies genetic exchanges among *E. coli* genomes from different ecological environments (3). Certainly not all pathogenicity traits are equally distributed among *E. coli* serotypes or STs. That probably means that a highly pathogenic clone emerges when an *E. coli* type able to sustain particular environmental interactions accumulates pathogenic genes. This is, in a sense, when virulence meets metabolism (4).

The strains belonging to the O104:H4 serotype (ST678, phylogroup B1), responsible for the severe German outbreak of bloody diarrhea and hemolytic-uremic syndrome in 2011, illustrate this concept. In their paper in *mBio*, "Comparative genomics of recent Shiga toxin-producing *Escherichia coli* O104:H4: short-term evolution of an emerging pathogen," Grad et al. (5) indicate that a rapid introgressive evolution has occurred in these strains by sequential acquisition of foreign genetic material, including such pathogenicity traits as Shiga toxin and aggregative-adherence fimbriae. The result is a highly pathogenic behavior for *E. coli* in humans, but what are the evolutionary benefits for the bacterial organism?

Such evolutionary benefits should exist. It seems likely that *E. coli* O104:H4 has undergone selection in some way or another in the recent past and has enlarged its population size and/or improved its adaptation to multiple habitats. The short-term evolution indicated by Grad et al. (5) and the cumulative acquisition of pathogenic traits require frequent and extensive ecological and genetic interactions with other bacterial (donor) cells, probably requiring a large number of cells and/or very effective dispersal, according to the genetic-capitalism principle (6). Studies based on whole-genome sequencing of several *E. coli* O104:H4 (ST678) strains isolated along the last few years revealed strong genetic differences in chromosomal and plasmid content (7). An unexpectedly high number of recombinant genes (125 genes) was found, and interestingly, the possible donors of these genes were not clustered in a single *E. coli* phylogenetic group. In fact, in half of cases, the recombinant genes contained sequences from donors in six phylogenetic groups. Even though the possibility of extensive recombination with a highly mosaic donor strain of another phylogroup cannot be totally excluded, differences among *E. coli* O104:H4 strains suggest separate sites and events in their recent evolutionary history. This implies a dense network of interactions with other bacteria. What could have been the necessary context for these interactions?

The simple answer is that organisms evolve from their natural reservoirs, where a sufficient population size can be reached. This natural reservoir, that is, the optimal environment for the reproduction, maintenance, and evolution of *E. coli* O104:H4, remains uncertain (8). *E. coli* is a normal component of the microbiotas of humans and animals, the natural place where *E. coli* strains might genetically interact with other *E. coli* strains and with many other *Proteobacteria*. Strains of serotype O104:H4 have been found mostly in association with sporadic cases of persistent or severe diarrhea since the 1990s, but little is known about their prevalence in healthy humans. Classic and modern studies from the 1960s indicate that O104 strains were scarcely found among normal hu-

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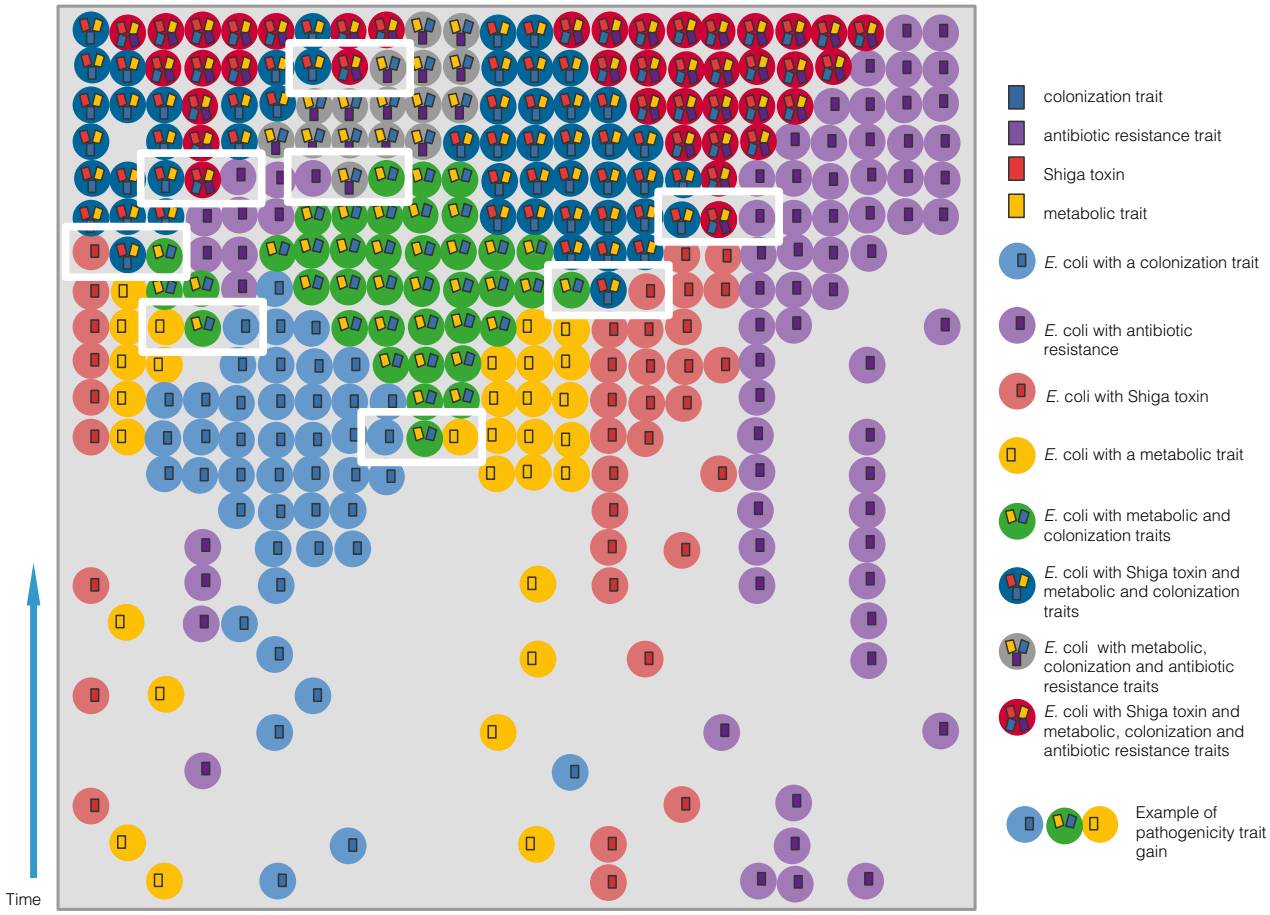

FIG 1 *E. coli* evolutionary networking with niche neighbors. Schematic space-time diagram illustrating the possible dynamics of the combinatorial evolution of pathogenicity traits (PTs) in *E. coli*. At the bottom of the figure, in light red, yellow, light blue, and violet, are the isolated strains containing single PTs. Such traits might increase the local spread of these strains; genetic interactions between pairs of them (white squares) produce novel strains with 2 PTs (the central box in the white square arises from the interaction of the flanking strains). Following new local spreads, strains with 2 PTs (like those in green), interact with other PT-containing strains, giving rise to strains with increasing numbers of PTs, resulting in multi-PT strains (shown in dark red, dark blue, and gray at the top of the figure) of different evolutionary trajectories.

man hosts at that time. It is worth noting that investigations during the outbreak in Germany also discarded the possibility of a cattle reservoir, unlike with other Shiga toxin-producing *E. coli* strains. However, the question of carriage merits further investigation. We agree with Grad et al. that the information about O104 epidemiology depends on the resolution of the technology used and the number of samples studied (9). At this time, the enemy-within-us hypothesis (10), that is, that humans are the only natural reservoir for *E. coli* O104:H4, should be seriously considered. It seems possible that humans can be colonized with such low numbers of the strain that they would be effectively invisible to classical culturing methods. This possibility is compatible with the observation of poor interhuman transmission among individuals in highly affected areas who share a household (11). These data seem to indicate that *E. coli* O104:H4 has gained little reproductive advantage from its amazing ability to collect pathogenic determinants, either in human hosts or in cattle.

The results presented in the Grad et al. work (5) require an understanding of the circumstances that allow fertile interactions between *E. coli* O104:H4 and other bacteria that may donate pathogenicity genes. Of course, big population sizes would facilitate these contacts, but it seems that O104:H4 does not form large populations. The evolutionary benefits of acquiring these donated traits are not expressed as high reproduction rates. However, that does not mean that O104:H4 lacks evolutionary advantages. *E. coli* O104:H4 might have evolved not as an *r*-strategist (oriented to maximal reproduction) but as a *K*-strategist (with lower numbers of individuals but higher efficiency in niche exploitation). Well established in its intestinal niche, and with a population density below the threshold for producing illness, *E. coli* O104:H4 might have had the opportunity of interacting with many other transient and resident *E. coli* strains and eventually with donors of pathogenicity traits. Donors of pathogenicity genes are probably available in human feces with a higher frequency than is usually assumed. Genes encoding one of the key pathogenicity traits of *E. coli* O104:H4, Shiga toxin (*stx* genes), were detected in Norway in 13% of healthy volunteers and in 36% of samples from patients (12). Each gain of new pathogenicity genes by *E. coli* O104:H4 contributes to a deeper exploitation of the intestinal niche. Note that along this process, a physical and functional convergence with other *E. coli* strains which have expanded their populations as a result of harboring colonization and/or antibiotic resistance traits

is expected to occur. Increased connectivity facilitates further "community genetic exchanges" building, as in the case of *Shigella* (an *E. coli* derivative), a quasi-species group (Fig. 1).

Other possibilities to explain the promiscuous life of *E. coli* O104:H4 in spite of its seemingly low population density remain to be explored. This strain was consistently found in a common food (salad) vehicle involved in the Germany and France outbreaks, fenugreek sprouts (*Trigonella foenum-graecum*, family Fabaceae), presumably of African origin. Might this legume, used on this continent both for animal pasture and for human food, be something more than a vehicle? Could O104:H4 be ecologically associated (and genetically interact) with the root nodule bacteria of this plant? The possibility of an endemic status of *E. coli* O104:H4 in humans in Central Africa has recently been suggested (8). Was this plant exposed during agricultural farming to animal and human sewage? We should clarify the field (network) of interaction giving rise to the outbreak strain.

As in a crowdsourcing software project, the evolution of O104:H4 starts with an initial plastic bacterium project onto which new elements, including pathogenicity genes, are anonymously contributed by similar bacteria. At the end, we have different combinations of elements that develop and grow continuously within the crowdsourcing community (Fig. 1). How to follow that process is the aim of predicting risks for future outbreaks.

Surveilling a reduced number of conserved genes can produce deceiving results, because pathogenicity genes are frequently allocated to mobile modules with little sequence conservation. The availability of next-generation sequencing (NGS) technologies allows us to reveal the complete gene landscape of a bacterium and, moreover, of a community of bacteria. This new perspective on outbreaks circumvents the old bias of looking only at specific conserved genes belonging to the causative agents. NGS enables scientists to trace the importance of gene units that move freely between strains and to analyze the flux of these genes between bacterial communities. In the postgenomics era, NGS technologies are providing public health efforts with advanced and quick tools that allow researchers not only to retrospectively analyze the epidemiological evolution of an outbreak but also to predict its future evolution.

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